



Resolving a paradox—high mercury deposition, but low bioaccumulation in northeastern Puerto Rico

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Abstract

At a “clean air” trade winds site in northeastern Puerto Rico, we found an apparent paradox: atmospheric total mercury (THg) deposition was highest of any site in the USA Mercury Deposition Network, but assimilation into the local food web was quite low. Avian blood THg concentrations ($n = 31$, from eight species in five foraging guilds) ranged widely from 0.2 to 32 ng g⁻¹ (median of 4.3 ng g⁻¹). Within this population, THg was significantly greater at a low-elevation site near a wetland compared to an upland montane site, even when the comparison was limited to a single species. Overall, however, THg concentrations were approximately an order of magnitude lower than comparable populations in the continental U.S. In surface soil and sediment, potential rates of demethylation were 3 to 9-fold greater than those for Hg(II)-methylation (based on six radiotracer amendment incubations), but rates of change of ambient MeHg pools showed a slight net positive Hg(II)-methylation. Thus, the resolution of the paradox is that MeHg degradation approximately keeps pace with MeHg production in this landscape. Further, any net production of MeHg is subject to frequent flushing by high rainfall on chronically wet soils. The interplay of these microbial processes and hydrology appears to shield the local food web from adverse effects of high atmospheric mercury loading. This scenario may play out in other humid tropical ecosystems as well, but it is difficult to evaluate because coordinated studies of Hg deposition, methylation, and trophic uptake have not been conducted at other tropical sites.

Keywords Methylmercury · Trophic uptake · Demethylation · Puerto Rico · Sulfate reduction · Iron reduction

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Introduction

Mercury (Hg) is a potent neurotoxin and a pervasive global pollutant that poses a risk to ecosystem and human health (Driscoll et al. 2013). Research on environmental cycling and trophic uptake of Hg has increased greatly over the last 25 years, but most research has focused on mid- and high-latitude ecosystems; Hg researchers have paid relatively scant attention to tropical landscapes (Sprovieri et al. 2017). The tropics merit greater attention because they are a reservoir for biodiversity and hold about half of the world's human population, including groups with diets high in fish, one of the main pathways of human exposure to Hg. The tropics may be a hotspot for Hg deposition from the global pool, even in the absence of point sources (Shanley et al. 2015).

Wet atmospheric Hg deposition in northeastern Puerto Rico, which receives trade winds from the northeast that cross mostly open ocean waters and is unimpacted by point sources, was higher than at any site in the continental USA

($28 \mu\text{g m}^{-2}$ annually in 2005–2007) (Shanley et al. 2015; Shanley et al. 2008). These studies concluded that high convective cloud tops that form over the island landmass scavenge gaseous oxidized mercury (GOM) from the large pool of GOM known to exist in the upper free troposphere (Landing et al. 2010, Lyman and Jaffe 2012, Swartzen-druber et al. 2006). Shanley et al. (2015) suggested that high Hg wet deposition could occur in other tropical settings where there is sufficient landmass to generate high convective cloud tops, but there are few data available.

The high Hg deposition was reflected in high stream-water Hg concentrations, mostly as particulate Hg, at Río Icacos, a high-elevation watershed (616–844 m) near the Hg deposition station. Stream particulate THg concentrations exceeded 100 ng L^{-1} and dissolved THg reached 5 ng L^{-1} during high flow periods. High Hg concentrations combined with high water flux in this rain forest landscape to yield an annual average stream THg flux at Río Icacos of $54 \mu\text{g m}^{-2} \text{ a}^{-1}$ (Shanley et al. 2008), more than an order of magnitude greater than typical temperate sites (Shanley and Bishop 2012). The stream THg flux thus is the same order of magnitude as wet and dry THg inputs. Thus, despite above average soil organic matter for a tropical forest (Johnson et al. 2015), the landscape is relatively ineffective at retaining Hg.

Most Hg studies in the tropics have focused on Hg-contaminated sites, such as gold mining and ore processing sites in the Amazon basin (Fostier et al. 2000; Fadini and Jardim 2001; Silva-Filho et al. 2006) and Guyana (Howard et al. 2011). Outside of Puerto Rico, only two studies have reported on systematic atmospheric deposition measurements for at least a full year: Barbados (Guentzel et al. 2001) and Mexico (Hansen and Gay 2013). A few tropical studies have reported avian Hg burdens: Hispaniola (Townsend et al. 2013), Nicaragua (Lane et al. 2013), Belize (Evers 2008), and Puerto Rico (Burger and Gochfeld 1991; Burger et al. 1992b), but these studies had no associated atmospheric deposition monitoring. Limited work in tropical Africa has focused on Hg in fish in lakes and a large inland delta wetland (Black et al. 2011 and references therein). Several authors have investigated Hg methylation processes in the freshwater tropics, especially in the Amazon basin (Achá et al. 2011; Guimarães et al. 2000; Lázaro et al. 2016; Mauro et al. 2002; Roulet et al. 2001), but these studies did not assess trophic uptake.

Despite the high deposition and mobility of Hg at our Puerto Rican site, two lines of evidence hinted that the Hg may have a limited effect on the ecosystem. First, a pilot survey of various aquatic and terrestrial biota suggested that Hg concentrations were less than those in comparable mid-latitude species (M. Bank and the authors, unpublished data). Secondly, only $\sim 0.3\%$ of THg was present as methylmercury (MeHg) in unfiltered streamwater, a

relatively low fraction (Shanley et al. 2008). The juxtaposition of high Hg deposition and low trophic uptake is not necessarily at odds, as inhibition of methylmercury formation will limit uptake (Wiener et al. 2006). However, landscape conditions at our site appeared to be conducive to methylation, including year-round warm temperatures (Murphy and Stallard 2012), above-average soil carbon (Johnson et al. 2015), ample sulfur availability (Peters et al. 2006), and occurrence of reducing microsites in surface soils (Hall et al. 2016). The apparent paradox prompted directed field campaigns to (i) confirm the preliminary finding of low trophic uptake by assessing Hg in avian blood, and (ii) investigate Hg(II)-methylation dynamics within soil and sediment. Our working hypothesis and rationale for this paper was that low net methylation was limiting trophic uptake despite high Hg deposition in this tropical wet forest. With the newly acquired information, we propose a reconciliation of the apparent paradox of biota that are exposed to high mercury loading but only sparingly bioaccumulate it.

Site description

The Luquillo Mountains are at 18°N in northeastern Puerto Rico, the easternmost island in the Greater Antilles, about 1600 km ESE of Miami, Florida (Fig. 1). Our research site was within the Luquillo Experimental Forest (LEF) in El Yunque National Forest. LEF is centered 35 km ESE of San Juan, and receives airflow off the Atlantic Ocean driven by northeasterly trade winds. The forest is mountainous, with peaks and ridges rising from sea level to 1075 m a.s.l. The Hg deposition station was in the northeastern part of the forest. Most of the soil, stream, and biota sampling took place in the Río Icacos basin, in the headwaters of Río Blanco, a few km to the SW of the deposition station on the south slope of the mountains (Fig. 1). Soils are sandy Inceptisols derived from the quartz diorite bedrock (Johnson et al. 2015). However, nearly all soils have aquic properties due to clay-rich B horizons that impede infiltration (Johnson et al. 2015).

Weather patterns that bring rainfall to Puerto Rico have marked seasonality (Scholl and Murphy 2014). Annual rainfall in the Luquillo forest averages 4200 mm (Murphy et al. 2017; Peters et al. 2006), with highest rainfall from April through December, and a distinctly drier January through March. The genesis of precipitation in winter and spring is trade wind-sea breeze showers from orographic uplift and frontal systems (Scholl and Murphy 2014). During summer and fall, the site receives rainfall from deep convection associated with tropical waves embedded in the prevailing easterly airflow. Tropical cyclones can produce heavy and intense rainfall. Mean annual temperature is 24°C with only a 4°C annual range in monthly averages.

Methods

Sediment and soil biogeochemistry

We conducted two discrete sampling and analysis campaigns—one for stream sediment and one for watershed soils (Fig. 2). In September 2008, as part of synoptic sampling of the five primary USGS Water, Energy, and Biogeochemical Budgets (WEBB) program locations (<http://water.usgs.gov/webb/>), we collected streambed surface sediment (top 0–2 cm) from three locations along a 2-km

reach of Río Icacos (Fig. 2). In February 2012, we sampled surface soils (0–10 cm) at three positions along a hillslope transect—upland (midslope), transition (toeslope), and a low-lying wetland area within the Río Icacos floodplain (floodplain). The hillslope transect represented a typical catena for this watershed, but the floodplain sampling site was unusual, as most of the channel is flanked by steep hillslopes. Two sets of duplicate soil samples were collected at each transect point. We focused on 0–10 cm soils for this study based on findings at a nearby site, where anaerobic respiration and potential Fe(III) reduction (factors potentially linked to Hg(II) methylation), were greatest in near-surface soils and decreased with depth as a consequence of C limitation (Hall et al. 2016). The hydrology and geomorphology of this catena are described in detail by McDowell et al. (1992).

Streambed sediment and one set of soil duplicates were transferred into acid-cleaned glass mason jars. All jars were filled to the top to exclude oxygen, stored chilled, and shipped overnight to the USGS laboratory in Menlo Park, California, for further sub-sampling under anoxic conditions (Marvin-DiPasquale et al. 2008) for methylation/demethylation incubations, and for several Hg and biogeochemical parameters (Bradley et al. 2011; Hall et al. 2013; Hall and Silver 2015; Marvin-DiPasquale et al. 2008) (Table 1). Sediment/soil parameters determined in Menlo Park included THg, MeHg, reactive Hg (Hg(II)_R), total reduced sulfur (TRS), microbial sulfate reduction rate (SRR), percent mass loss-on-ignition (%LOI), and solutes in 0.45- μm -filtered porewater (collected by centrifugation) (Table 1).

The second set of replicate soil samples was collected primarily to determine Fe speciation. Extractions were initiated in the field and completed within two hours to minimize potential changes in soil Fe speciation associated with changes in redox conditions. Soil subsamples were extracted in solutions of 0.5 M HCl (1:10 ratio, soil:

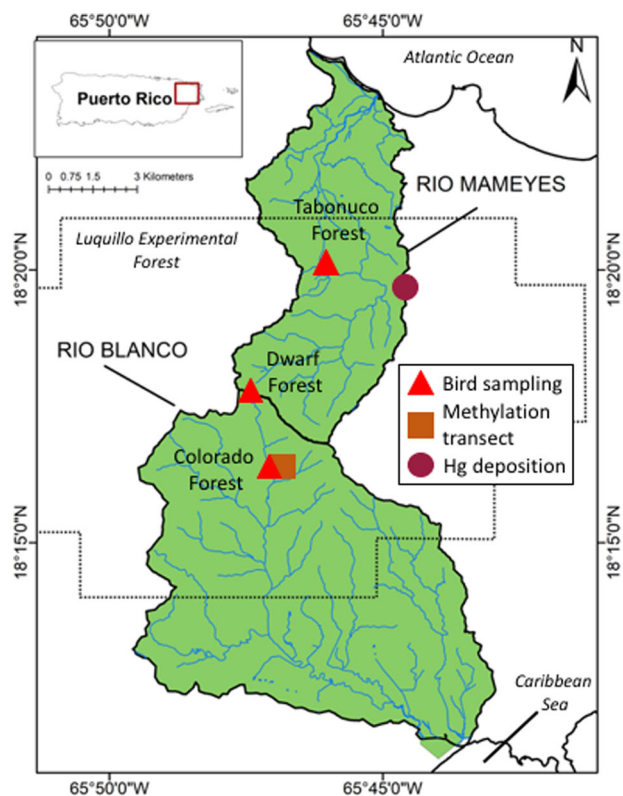


Fig. 1 Map of sampling sites and location within Puerto Rico

Fig. 2 **a** MeHg concentrations in soils (topmost 10 cm) and streambed sediment (topmost 2 cm) along a transect from hillslope to stream; **b** MeHg vs. loss-on-ignition (LOI) in soil and streambed sediments. LOI was used because %C was not measured on streambed sediment (but see Fig. A-1)

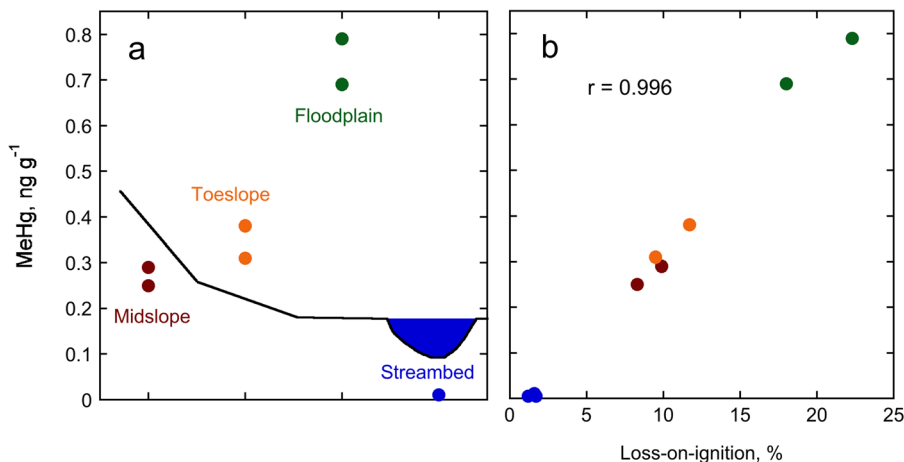


Table 1 Summary of soil and streambed sediment Hg metrics and relevant biogeochemistry for Río Itacos

Notation	Parameter	Units	Midslope soil		Toe of slope soil		Floodplain soil		Stream bed sediment		Reference
			Mean	Dev.	Mean	Dev.	Mean	Dev.	Mean	Std. Dev.	
<i>Mercury parameters</i>											
THg	Total mercury	(ng/g) dry wt.	48.52	2.26	51.34	7.36	127.60	15.65	2.93	1.34	Marvin-DiPasquale et al. 2011
MeHg	Methylmercury	(ng/g) dry wt.	0.27	0.02	0.34	0.03	0.74	0.05	<0.01		Marvin-DiPasquale et al. 2011
MeHg/THg (%MeHg)	percent methylmercury	%	0.34	0.01	0.41	0.05	0.28	0.03	<0.32		calculated
Hg(II) _{org}	inorganic reactive mercury	(ng/g) dry wt.	3.75	0.43	3.72	1.59	3.07	1.82	0.21	0.15	Marvin-DiPasquale et al. 2011
Hg(II) _R /THg (%Hg(II) _R)	percent reactive mercury	%	7.80	1.26	6.95	2.10	2.26	1.15	6.71	1.97	calculated
K _{meth}	MeHg production potential rate constant	(1/d)	<0.001		<0.001		0.002	0.001	<0.001		Marvin-DiPasquale et al. 2011
K _{deg}	MeHg degradation potential rate constant	(1/d)	0.055	0.005	0.037	0.014	0.041	0.025			see text
MPP	MeHg production potential rate (calculated)	(pg/g dry sed/d)	<3.72		<3.62		4.49	0.91	<0.10		Marvin-DiPasquale et al. 2011
MDP	MeHg degradation potential rate (calculated)	(pg/g dry sed/d)	14.26	2.49	12.90	5.87	30.67	19.54			calculated from k _{deg} and native MeHg concentration, akin to Marvin-DiPasquale and Agee 2003
NMP	Net MeHg production, based on change in native Me ²⁺ Hg	(pg/g dry sed/d)	12.76	7.43	15.99	4.54	18.52	13.79			See text
<i>Non-Mercury Parameters</i>											
%dry wt.	Percent dry weight	%	62.6	0.6	55.8	4.2	47.1	1.9	76.0	1.6	Marvin-DiPasquale et al. 2008
BD	Wet bulk density	(g/cm ³)	1.51	0.02	1.47	0.06	1.30	0.02	1.81	0.09	Marvin-DiPasquale et al. 2008
POR	Porosity	(ml porewater/cm ³)	0.57	0.00	0.59	0.04	0.69	0.01	0.43	0.01	Marvin-DiPasquale et al. 2008
%LOI	Percent Weight Loss on ignition	(% of dry wt.)	9.15	0.80	10.59	1.12	20.14	2.12	1.47	0.25	Marvin-DiPasquale et al. 2008
%C	Percent Organic carbon	(% of dry wt.)	2.70	0.30	3.10	0.40	8.50	2.00			Hall and Silver 2015
%fines (silt and clay)	Percent Grain Size (% <63 micron)	(% <63 um)	40.87	2.60	36.30	1.15	77.28	7.00	1.43	0.57	Matthes et al. 1992
E _h (laboratory)	Oxidation-reduction potential	(mv)	+561	12	+527	43.00	+459	117	+497	27	Marvin-DiPasquale et al. 2008
pH (field)	pH	(pH units)	3.35	0.04	3.47	0.27	3.80	0.06	6.37	0.02	Marvin-DiPasquale et al. 2008
SRR	Microbial sulfate reduction rate	(mmol/g/d) dry wt.	0.02	0.01	0.04	0.04	0.60	0.10	0.05	0.01	Marvin-DiPasquale et al. 2008
TRS	Total reduced sulfur	(umol/g) dry wt.	0.23	0.06	0.30	0.04	0.87	0.26	0.16	0.06	Marvin-DiPasquale et al. 2008
Fe(II) _{HCl}	Ferrous Iron, HCl extractable	(mg/g) dry wt.	0.15	0.01	0.18	0.10	0.22	0.00	0.13	0.02	Hall et al. 2016
Fe(II) _{HCl}	Ferrous Iron, HCl extractable	%	1.55	0.02	1.15	0.69	0.44	0.24	11.84	6.75	Hall et al. 2016
%Fe(II) _{HCl}	Ferrous Iron, percent HCl extractable	%	9.01	0.75	14.09	0.80	38.65	14.42	1.48	0.97	Calculated
Fe(II) _{org}	Ferrous Iron, short-range-ordered ^a	(mg/g) dry wt.	17.35	8.56	5.68	2.75	1.31	0.70			Hall et al. 2016
Fe(II) _{iso}	Ferrous Iron, crystalline ^b	(mg/g) dry wt.	19.94	10.30	5.81	5.71	0.06	0.03			Hall et al. 2016
%Fe(II)/Fe ^c	Ferrous iron, percent of total extractable Fe ^c	%	0.41	0.05	2.26	0.87	17.02	7.30			Calculated
CH ₄ flux	CH ₄ flux	umol m ⁻² h ⁻¹	<5		<5		29.50	3.50			Hall et al. 2013
<i>Porewater chemistry^d</i>											
[SO ₄ ²⁻]	Sulfate	(umol/liter)	114.08	58.92	77.33	1.06	49.73	30.15	22.24	4.09	Marvin-DiPasquale et al. 2008
[Cl ⁻]	Chloride	(umol/liter)	0.78	0.28	0.50	0.03	0.48	0.20	0.19	0.02	Marvin-DiPasquale et al. 2008
[SO ₄ ²⁻]/[Cl ⁻]	Sulfate to chloride molar ratio	Unitless	0.14	0.03	0.15	0.01	0.16	0.13	0.11	0.01	Calculated
[DOC]	Dissolved organic carbon	(mg/liter)	93.80	50.50	97.18	10.77	37.35	0.81	7.57	3.09	Marvin-DiPasquale et al. 2009a

Soil samples are 0–10 cm depth and streambed sediments are 0–2 cm depth. Soil values based on duplicate samples along a hillslope to flood plain transect; sediment values based on three samples along a 2-km stream reach adjacent to the soil sampling transect. For soils (Midslope, Toe of slope and Floodplain), the error term reflects the deviation of n = 2 samples (DEV = (X1 – X2)/2). For the stream samples, the error term reflects the standard deviation (STDEV) of n = 3 samples

^aFe(III)_{org} = [citrate-ascorbate extractable Fe] – Fe(II)_{HCl}

^bFe(III)_c = [citrate-dithionite extractable Fe] – [citrate-ascorbate extractable Fe]

^c%Fe(II)_T = Fe(II)_{HCl}/[citrate-dithionite extractable Fe] × 100

^dPore water was collected from elutriated samples, to generate sufficient aqueous volume for all analyses

extractant) and 0.2 M sodium citrate/0.05 M ascorbic acid (1:30 ratio, soil:extractant). HCl extractable Fe fractions included both ferrous iron ($\text{Fe(II)}_{\text{HCl}}$) and ferric iron $\text{Fe(III)}_{\text{HCl}}$. The citrate-ascorbate Fe fraction was used to calculate short-range-ordered Fe (Fe_{SRO} , Table 1), as modified from Hall and Silver 2015). Dried and ground subsamples were extracted in a citrate-dithionite solution (1:60 ratio, soil:extractant) to determine total extractable Fe (Fe_{T}), from which crystalline Fe ($\text{Fe(III)}_{\text{C}}$) was calculated by subtracting Fe_{SRO} (Table 1). We determined percent carbon (%C: assumed equal to % organic carbon based on soil acidity) on these samples by combustion on an elemental analyzer, and we also measured soil-atmosphere methane (CH_4) fluxes via static chambers (following Hall et al. 2013) at each soil-sampling site as an additional proxy for anaerobiosis. See supplementary OR material for detail on the trace gas sampling.

Hg methylation and demethylation incubations

Incubations were initiated within 24–48 h of sampling. For both streambed sediments and soils, methylmercury production rate constants (k_{meth}) and calculated potential (MPP) rates were assessed via stable isotope ($^{200}\text{Hg}^{2+}$) amendment with 22-hr bottle incubations (streambed sediment) or 5-hr bottle incubations (soils). The shorter incubation time for soils was in anticipation of higher MPP rates. Each site-specific sample set consisted of duplicate incubated samples plus one killed control (flash frozen immediately after isotope amendment). The final $^{200}\text{Hg(II)}$ amendment concentration was approximately 50 ng g^{-1} sediment wet weight, which for the three Río Icacos stream sites was equivalent to $66\text{--}69 \text{ ng g}^{-1}$ dry wt., or more than an order of magnitude higher than the native THg concentration for these sandy stream samples ($1.6\text{--}4.2 \text{ ng g}^{-1}$ dry wt). For the soils, the final $^{200}\text{Hg(II)}$ amendment concentration was $74\text{--}105 \text{ ng g}^{-1}$ dry wt., which was quite similar to the native THg concentration ($44\text{--}144 \text{ ng g}^{-1}$ dry wt.). Because $^{200}\text{Hg(II)}$ amendments were not at trace levels relative to native THg pools, the MPP incubation should be regarded as a ‘potential rate’ determination. The resulting end-product (Me^{200}Hg) was quantified by isotope dilution ICP-MS (Marvin-DiPasquale et al. 2011). Final concentrations were determined for each sample by subtracting the (nearly negligible) killed-control value for each site from the two incubation values.

In like manner, we assessed MeHg degradation rate constants (k_{deg}) and calculated mercury degradation potential (MDP) rates on soils only by incubating for six days after amendment with Me^{201}Hg ($1.6\text{--}2.2 \text{ ng g}^{-1}$ dry wt., compared to native MeHg concentrations of $0.2\text{--}0.8 \text{ ng g}^{-1}$ dry wt.), and determining rates from the loss of Me^{201}Hg over time. The longer incubation time improves the chance

of detecting loss, which is determined by difference. In addition, we tracked the concentration change in the native Me^{202}Hg pool in the MDP samples as a measure of net MeHg production (NMP) (net degradation if negative), as this isotope was not added as an enriched amendment.

Avian blood sampling

During four days in May 2010 we captured 31 birds for blood Hg analysis by opportunistic mist netting in three forest types: elfin woodland near the summit of Mt. Britton ($n = 2$); high-elevation palo colorado forest near Río Icacos gage ($n = 17$); and low-elevation tabonuco forest near El Portal ($n = 12$) (Fig. 1). See McDowell et al. (2012) for a detailed description of these habitats. We did not target particular species, but were fortunate to capture birds representing all five feeding guilds, and all but one individual, a black-whiskered vireo (*Vireo altiloquus*), were permanent residents. Of the 31 birds, 19 were bananaquits (*Coereba flaveola*), a small non-migratory nectarivore that also consumes flies, beetles, caterpillars, ants, bees, and spiders (Allen 1961; Stiles and Skutch 1989), mostly from the undersides of leaves (Cruz 1980). The remaining 12 birds represented seven species, with no more than 3 individuals of any species. Blood was collected non-lethally from the brachial vein using 27-gauge disposable needles and heparin-coated capillary tubes, stored in labeled vacutainers, and frozen within four hr of collection. Birds were handled under appropriate permits and released unharmed at the capture site.

We used the nonparametric Proportions (z-test) and Mann–Whitney Rank Sum Test (U statistic) to test for differences in Hg among bird populations. We compared blood THg concentrations of bird populations in the low-elevation tabonuco forest ($n = 17$) and the high-elevation palo colorado forest ($n = 12$) (for this comparison we excluded the two birds captured in elfin woodland). To avoid interspecies differences, we also compared blood THg in populations of bananaquit in the low-elevation ($n = 8$) and high-elevation ($n = 10$) groups.

Because Hg in bird blood is nearly 100% MeHg (Edmonds et al. 2010; Rimmer et al. 2005), blood was analyzed for THg only. THg was determined from the entire sample volume using USEPA method 7473 (EPA 1998) by gold-amalgamation atomic absorption spectroscopy following thermal desorption. The analytical instrument used was a Milestone DMA-80 at Biodiversity Research Institute (BRI), Wildlife Mercury Research Laboratory (WMRL) in Portland, Maine. Internal lab quality control included initial calibration and continuing verification, blanks, sample replication, and analysis of the commonly used certified reference materials DORM-3 and DOLT-4.

Blood samples were also analyzed for stable isotopes of C and N at the Boston University Stable Isotope Laboratory using automated continuous-flow isotope ratio mass spectrometry (Michener and Lajtha 2007). The samples were combusted in a EuroVector Euro EA elemental analyzer. The combustion gases (N_2 and CO_2) were separated on a GC column, passed through a reference gas box and introduced into a GV Instruments IsoPrime isotope ratio mass spectrometer; water was removed using a magnesium perchlorate water trap. Ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ are reported as standard delta (δ) notation and are expressed as the relative permil (‰) difference between the samples and international standards (Vienna Pee Dee Belemnite (V-PDB) carbonate and N_2 in air):

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000(\text{‰})$$

where $X = ^{13}\text{C}$ or ^{15}N and $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The sample isotope ratio was compared to a secondary gas standard, the isotope ratio of which was calibrated to international standards. For $^{13}\text{C}_{\text{V-PDB}}$ the gas was calibrated against NBS 20 (Solenhofen Limestone). The $^{15}\text{N}_{\text{air}}$ gas was calibrated against atmospheric N_2 and International Atomic Energy Agency (IAEA) standards N-1, N-2, and N-3 (all were ammonium sulfate standards).

Results

Mercury and relevant biogeochemistry of streambed and soil

The downslope soil transect, from midslope to toeslope to floodplain, exhibited a progressive decrease in soil % dry weight (63–47%), and wet bulk density ($1.51\text{--}1.30\text{ g/cm}^3$), and a progressive increase in soil porosity (0.57–0.69), organic content (as %LOI, 9.1–20.1%), %fines (silt + clay; 40 to 77%), and pH (3.35–3.80) (mean values; Table 1). By comparison, the sandy (quartz diorite bedrock dominated) Río Icacos streambed sediment was the most elevated in % dry weight (76%), wet bulk density (1.81 g/cm^3), and pH (6.37), while having the lowest porosity (0.43), %LOI (1.5%), and %fines (1%). Across all sites, oxidation-reduction potentials (as E_h) at the time of sampling were notably oxalic (+400 to +500 mv).

Mean THg concentrations were lowest in the streambed ($2.9 \pm 1.3\text{ ng g}^{-1}$) and much higher in soils, from $50 \pm 6\text{ ng g}^{-1}$ in hillslope soils ($n = 4$ sites, mid- and toeslope) to $128 \pm 16\text{ ng g}^{-1}$ in the floodplain ($n = 2$ sites). MeHg concentrations were at or below detection ($\leq 0.01\text{ ng g}^{-1}$ dry wt.) in the streambed, $0.31 \pm 0.05\text{ ng g}^{-1}$ ($n = 4$) in the hillslope, and $0.74 \pm 0.05\text{ ng g}^{-1}$ ($n = 2$) in the floodplain (Fig. 2, Table 1). These values equated to $<0.4\%$ MeHg in the

streambed and ranged from 0.55 to 0.68% MeHg in soils. As a percentage of THg, Hg(II)_R was lowest in the floodplain ($2.3 \pm 1.1\%$) ($n = 2$) compared to all other soil and sediment sites ($7.1 \pm 1.9\%$, $n = 7$) (Table 1). THg, MeHg, %fines, and %LOI were all strongly and positively inter-correlated, with Pearson r values ranging from 0.987 to 0.998 for the pairwise combinations (Fig. 2; Figure OR1).

Considering the well-oxygenated status of the soils and sediments sampled, it is not surprising that the biogeochemical metrics associated with microbial sulfate reduction all suggested minimal to no activity for this terminal heterotrophic anaerobic pathway. Specifically, SRRs associated with anoxic bottle incubations were very low in stream bed sediment ($0.05 \pm 0.01\text{ nmol g}^{-1}\text{ d}^{-1}$, $n = 3$) and hillslope soils ($0.03 \pm 0.03\text{ nmol g}^{-1}\text{ d}^{-1}$, $n = 4$) and higher but still low in the floodplain ($0.60 \pm 0.10\text{ nmol g}^{-1}\text{ d}^{-1}$, $n = 2$). Bulk substrate TRS was also lowest for the streambed ($0.16 \pm 0.01\text{ } \mu\text{mol g}^{-1}$, $n = 3$) and hillslope soils ($0.27 \pm 0.07\text{ } \mu\text{mol g}^{-1}$, $n = 4$) and low for floodplain sites ($0.87 \pm 0.26\text{ } \mu\text{mol g}^{-1}$, $n = 2$). Further, porewater $\text{SO}_4^{2-}/\text{Cl}^-$ molar ratios showed little difference among all sites (means ranged from 0.11 ± 0.01 for streambed sediment to 0.16 ± 0.13 for the floodplain), suggesting minimal differences in SO_4^{2-} depletion, relative to the conservative anion Cl^- , among the various soil/sediment environments. Finally, free sulfide was not measured in the soil samples but was below detection ($<0.2\text{ } \mu\text{mol L}^{-1}$) in the streambed samples.

In contrast to sulfur cycling, metrics associated with microbial iron redox cycling suggest a more active terminal heterotrophic pathway across the study sites. While concentrations do not represent rates, the relative pools of Fe(II) and Fe(III) give us some sense of a system poised for Fe cycling, with spatially consistent variation along the study transect. Specifically, the proportion of 0.5 M HCl-extractable Fe in the reduced Fe(II) form ($\%\text{Fe(II)}_{\text{HCl}}$) was lowest in the streambed ($1.5 \pm 1.0\text{ mg g}^{-1}$, $n = 3$) and increased from the hillslope ($11.6 \pm 3.1\text{ mg g}^{-1}$, $n = 4$) to the floodplain ($38.6 \pm 14.4\text{ mg g}^{-1}$, $n = 4$), suggesting increasing microbial Fe(III) reduction from the streambed to the hillslope to the floodplain. Similarly, the proportion of Fe(II) relative to the total extractable Fe pool (via citrate-dithionite extraction) showed a similar progressive increase in $\%\text{Fe(II)}/\text{Fe}_T$ from midslope ($0.4 \pm 0.0\text{ mg g}^{-1}$, $n = 2$), to toeslope ($2.3 \pm 0.9\text{ mg g}^{-1}$, $n = 2$), to floodplain ($17.0 \pm 7.3\text{ mg g}^{-1}$, $n = 2$) (streambed not sampled). Supporting the Fe(II) distribution, patterns in CH_4 fluxes from soils to the atmosphere provided complementary evidence for an increase of anaerobic microsites (i.e., conditions suitable for Hg methylation) from upslope to the floodplain. CH_4 fluxes were not different from zero ($<5\text{ } \mu\text{mol m}^{-2}\text{ h}^{-1}$) at the midslope and toeslope sites, but floodplain soils had net positive CH_4 emissions of $26\text{--}33\text{ } \mu\text{mol m}^{-2}\text{ h}^{-1}$.

Methylation and demethylation incubations

Average MeHg production rate constants (k_{meth}), measured via incubation with the ^{200}Hg amendment, were below detection (<0.001) at all sites except the floodplain ($0.002 \pm 0.001 \text{ d}^{-1}$), as were subsequently calculated MPP rates at all sites ($<4.0 \text{ pg g}^{-1} \text{ d}^{-1}$) except the floodplain ($4.5 \pm 0.9 \text{ pg g}^{-1} \text{ d}^{-1}$) (Fig. 3, Table 1). MPP rates should be considered as an upper limit, as the initially oxic samples were incubated under anoxic conditions. In contrast, MDP rates, measured in soils only, ranged from 7–50 $\text{pg g}^{-1} \text{ d}^{-1}$, approximately 2 to 10 times higher than the corresponding MPP rates.

NMP rates, based on 6-day anoxic incubations and the change in the native Me^{202}Hg pool, were low and generally increased downslope, but were not significantly different among sites (Table 1: midslope, $12.8 \pm 7.4 \text{ pg g}^{-1} \text{ d}^{-1}$; toeslope, $16.0 \pm 4.5 \text{ pg g}^{-1} \text{ d}^{-1}$; floodplain, $18.5 \pm 13.8 \text{ pg g}^{-1} \text{ d}^{-1}$).

Bird blood Hg concentrations

Total blood Hg concentrations were quite low, ranging from 0.2 to 32 ng g^{-1} wet weight, with a median of 4.3 ng g^{-1} (Fig. 4; Table OR1). Within the total sample population ($n=29$, excluding the two elfin forest birds), Hg concentration was significantly greater (Proportions test, $p=0.013$) in the low-elevation tabonuco forest (83% of birds greater than overall population median Hg concentration) relative to the high-elevation palo colorado forest (29% of birds greater than overall population median Hg concentration). Bird blood Hg in the 19 Bananaquits nearly spanned the range for the entire 31-bird sample population and was not different from the overall population (Proportions test, $p=0.663$). Three species, with just one individual each, were either omnivorous (pearly-eyed thrasher (*Margarops fuscatus*) and red-legged thrush (*Turdus plumbeus*), both of which consume insects, land crabs, lizards, frogs, birds, and baby rats) or insectivorous (black-whiskered vireo). Despite these three individuals occupying higher trophic level feeding guilds, only the thrush was considerably above the population median (13.0 vs. 4.3 ng g^{-1}).

To minimize the interspecies effect, we compared bananaquit populations in the two forest types. As with the overall population, bananaquits had significantly higher blood Hg (Mann–Whitney Rank Sum Test, $p=0.009$) in the low-elevation tabonuco forest (median 8.1 ng g^{-1}) than in the high-elevation palo colorado forest (median 3.1 ng g^{-1}). The two birds from the high-elevation elfin woodland site (a bananaquit and a Puerto Rican bullfinch (*Melopyrrha portoricensis*)) had blood THg concentrations well below the overall sample population median of 4.3 ng g^{-1} . Overall, Hg concentration in bird blood had a weak but significant positive correlation with $\delta^{15}\text{N}$ (Fig. 4; Table OR1).

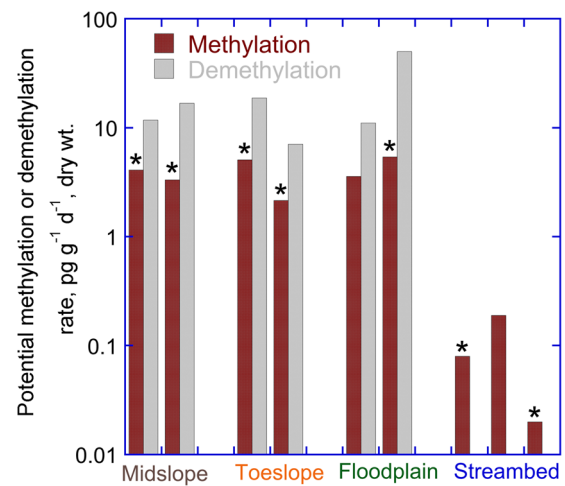


Fig. 3 Potential methylation and demethylation rates in soils and potential methylation rates in streambed sediment. Most of the methylation rates shown are the detection limits (marked by star) and should be considered as an upper bound to the potential rates

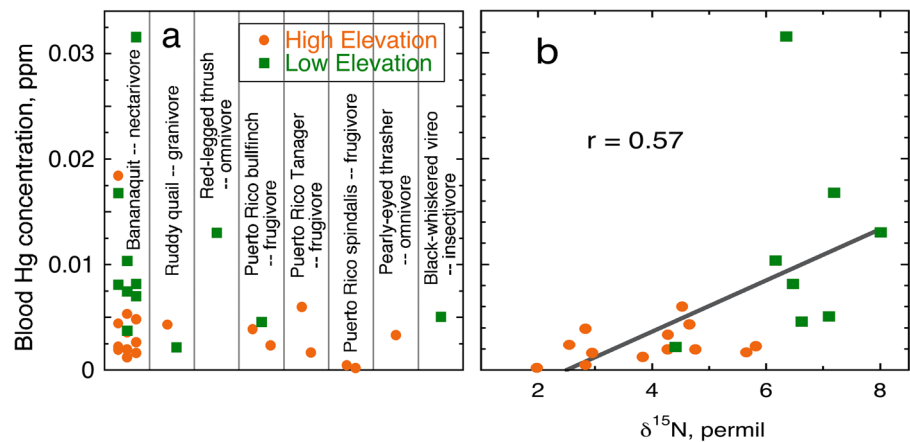
Discussion

Hg stocks, fluxes, and speciation at LEF

Given the high Hg wet deposition in this tropical wet forest that receives 2–5 m of rain per year, the relative importance of dry Hg deposition was an open question. Dry deposition of Hg in forested landscapes typically is 2–3 times wet deposition (St. Louis et al. 2001). Measurements from three years of litterfall in the Luquillo forest indicate that dry deposition was 1.5 times wet deposition (Risch 2017). Thus, the combined wet plus dry Hg deposition was quite elevated. The percentage of THg as MeHg (%MeHg) averaged only 0.06% in wet deposition (R. Brunette, Eurofins Scientific, unpubl. data, 2012) and 0.31% in litterfall (Risch 2017). These %MeHg values are well below global medians of 1.14% for wet deposition and 1.08% for litterfall (calculated from Shanley and Bishop (2012)).

Forested landscapes typically retain about 90% of atmospheric THg inputs, leaving 10% or less to run off in streamflow (Shanley and Bishop 2012). The portion of Hg that does not run off is retained in watershed soils and vegetation, and some Hg may also volatilize to the atmosphere (Krabbenhof et al. 2005). At LEF, Río Icacos had an unusually low watershed retention (high stream THg export) (see tabulation in Shanley and Bishop (2012)), but stream Hg export may have been augmented by legacy Hg from gold ore processing in the watershed more than a century ago (Wardsworth 1949). Stream Hg export was dominated by particulate Hg. As in rainfall and throughfall, a low percentage of THg was in the MeHg form; %MeHg in whole water samples, generally dominated by particulate Hg, was 0.68% (Shanley et al. 2008), similar to %MeHg in

Fig. 4 **a** Bird blood Hg concentration in 31 birds sampled in Luquillo Experimental Forest; **b** relation of bird blood Hg concentration to $\delta^{15}\text{N}$ in these samples



soils. The corresponding global median percentage was 2.8% (calculated from Shanley and Bishop (2012)).

In soils and sediment, the distribution of THg across the sites was highly correlated with grain size (as %fines, $r = 0.98$) and organic content (as %LOI, $r = 0.99$), and MeHg concentration was also positively correlated with THg ($r = 0.99$) (Figure OR1), with all of these metrics most elevated at the floodplain site and lowest in the streambed sediment. Given the low number of observations ($n = 9$, including replicates), a high degree of co-correlation is not surprising. Anecdotally, we previously measured high MeHg concentrations of 7.7 ng L^{-1} (MeHg/THg = 3.3%) in organic-rich water in the epiphytic tank bromeliad at LEF (Shanley et al. 2008), suggesting that Hg, S, and organic C for Hg(II)-methylation were amply supplied from the atmosphere and forest canopy and were not limiting Hg(II)-methylation.

A less commonly measured Hg metric is the Hg(II)_{R} fraction, which has been assessed in a number of studies as a proxy for chemically labile and microbially available Hg (II), and is used here and elsewhere (Marvin-DiPasquale et al. 2009a; Marvin-DiPasquale et al. 2009b; Marvin-DiPasquale et al. 2014) to calculate MPP rates (in combination with independently measured values of k_{meth}). Concentrations of Hg(II)_{R} increased with substrate redox potential (E_{h} , $r = 0.85$) and % Hg(II)_{R} decreased with increasing TRS concentration ($r = -0.76$), microbial SR rate ($r = -0.72$) and k_{meth} ($r = -0.71$), similar to patterns reported in earlier research (Marvin-DiPasquale et al. 2009a, Marvin-DiPasquale et al. 2009b, Marvin-DiPasquale et al. 2014).

Trophic Hg uptake

Avian Hg blood concentration in Puerto Rican birds was strikingly low (Fig. 5). The Puerto Rican birds that we were able to capture by mist netting included mostly frugivores and nectarivores, which would be expected to have low body burden Hg, but their blood Hg levels were still at least

a factor of five lower and generally an order of magnitude lower than birds in comparable feeding guilds at other tropical and temperate sites (Fig. 5) (Cristol et al. 2008; Evers and Duron 2006; Jackson et al. 2015; Lane et al. 2013). The extent of Hg bioaccumulation in the Puerto Rican birds followed the typical pattern of increase with increasing $\delta^{15}\text{N}$ (Fig. 4), a proxy for relative position in the feeding guilds (Cabana and Rasmussen 1994).

Avian blood Hg concentrations in Puerto Rico were anomalously low not only compared to mainland U.S. birds, but also compared to other tropical sites, including Belize, Nicaragua, and Hispaniola (Fig. 5). In Belize, more than half of 31 species of songbirds sampled had more than an order of magnitude higher Hg than the Puerto Rican birds (Evers 2008). Birds in Nicaragua had on average five times the blood Hg as in our Puerto Rican study, with 71 of 73 individuals having greater blood Hg than the median value of the 31 birds in Puerto Rico (Lane et al. 2013). In Hispaniola, Townsend et al. (2013) found a strong elevational gradient of blood Hg concentrations, with generally much lower values in birds in the lowland (near sea level) rain forest relative to the high-elevation cloud forest. Among the 13 sites sampled, birds from high-elevation cloud forest sites (up to 1800 m) had generally an order of magnitude higher Hg in their blood than at lowland sites, though the latter still had several-fold higher Hg than the Puerto Rican birds. Newer 2017 results (unpublished data available from co-authors Oksana Lane, Biodiversity Research Institute, Portland, ME, and Wayne Arendt, U.S. Forest Service, Luquillo, PR), however, show comparable or even lower bird blood Hg for bananaquits in city parks of coastal Santo Domingo than in the Puerto Rican mountains.

Though our most-sampled bird was the generally nectarivorous bananaquit, two lines of evidence suggest that bananaquits in LEF rely considerably on animal food sources and thus their low blood Hg is not solely a reflection of low trophic position. LEF is frequently impacted by tropical cyclones, which limit energetically expensive

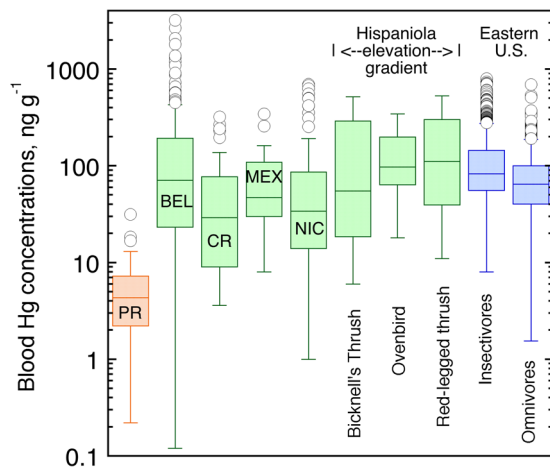


Fig. 5 Hg concentrations in the Puerto Rico birds sampled in this study compared to Hg concentrations in birds at other tropical sites and the northeastern USA. Orange: this study (PR = Puerto Rico). Green: other Caribbean and Central American sites (BEL = Belize, Evers 2008; CR Costa Rica, Biodiversity Research Institute (BRI), Portland Maine, unpublished data; MEX = Mexico, BRI, unpublished data; NIC Nicaragua, Lane et al. 2013; Hispaniola data for 3 species populating the majority of sites along an elevational transect (Townsend et al. 2013). Blue: northeast USA temperate sites (Jackson et al. 2015)

nectar production, as was found at similar elevations in other Puerto Rican forests (Ackerman et al. 1994; Meléndez-Ackerman et al. 2000; Pérez et al. 2011). In Jamaica's Blue Mountains at a similar elevation to our study, Cruz (1980) found that invertebrates constituted 24% of the bananaquit diet. Secondly, the three LEF species of hummingbirds, which rely more strongly on nectar, collectively have only 3.2% the relative abundance of bananaquits at LEF (unpublished data available from co-author Wayne Arendt, U.S. Forest Service, Luquillo, PR). In contrast, the two hummingbird species in Santo Domingo, where nectar is plentiful from the urban ornamental plants and introduced tree and shrub species which constitute 71% of the city's arboreal species (Melendez-Ackerman et al., University of Puerto Rico at Río Piedras, unpubl. manuscript), have nearly 30% the relative abundance of bananaquit. More detail on the bananaquit diet is available in the supplemental OR material.

Aside from bananaquits, the only species common to sampling efforts in Puerto Rico and Hispaniola was the red-legged thrush. Only one individual was captured in Puerto Rico, but its blood Hg matched the very lowest Hg concentration of the 41 red-legged thrushes captured by Townsend et al. (2013) in Hispaniola (Fig. 5). The elevational gradient in Hispaniola was reversed in Puerto Rico, where birds had significantly higher Hg at a lower elevation site, possibly due to a more complex food web and presence of a nearby wetland. These differences within and across regions underscore the important role of habitat in trophic

Hg uptake. But it is difficult to evaluate habitat effects without information on Hg deposition. As Townsend et al. (2013) noted for the Hispaniola study, atmospheric Hg deposition monitoring is a critical missing link that makes it difficult to distinguish the effects of Hg deposition from diet and other factors.

The literature on biotic uptake of Hg in Puerto Rico is limited, but some studies from the early 1990s assessed Hg in fauna in nearby coastal areas of Puerto Rico. Burger et al. (1992a) reported Hg lower than global averages in a variety of biota in and adjacent to freshwater-dominated coastal salt marshes in eastern Puerto Rico, although they had a high detection limit of 80 ng g⁻¹. Similarly, Burger et al. (1992b) found that cattle egrets (*Bubulcus ibis*) in eastern Puerto Rico had lower Hg than their urban counterparts. In contrast, Burger and Gochfeld (1991) found that Hg in tern feathers on the island of Culebra, Puerto Rico, was comparable or higher than in more urbanized areas. The relatively lower Hg in the terrestrial egret is consistent with our results, whereas Hg in terns is controlled by marine processes.

Some studies of Hg in fish in Puerto Rico run counter to our finding of low trophic uptake at LEF, with Hg concentrations near consumption advisory levels (Mansilla-Rivera and Rodríguez-Sierra 2011, Ortiz-Roque and López-Rivera 2004; Sastre et al. 1999), but these results are either from marine or contaminated sites. On the other hand, fish Hg in tropical Africa is well below global averages in lakes and the large inland Okavango delta wetland, leading Black et al. (2011) to refer to the "tropical African mercury anomaly". Black et al. (2011) did not resolve the anomaly, but discounted the lack of Hg in atmospheric deposition, pointing out that modeled Hg deposition on the African continent was higher than the global average (Selin et al. 2008). While the African anomaly mirrors our Puerto Rican paradox, a lake in Papua New Guinea (Bowles et al. 2001) and the Rio Negro in Brazil (Belger and Forsberg 2006) had much higher fish Hg.

Hg methylation and demethylation on the landscape

All indicators of methylation in soils and sediment tested in the LEF ecosystem hovered near our limits of detection. These include MeHg concentration itself, MPP, and the k_{meth} values on which MPP is partly based. MeHg concentrations averaged more than an order of magnitude lower than those in California wetlands (Marvin-DiPasquale et al. 2014) and a diversity of eight stream sites across the contiguous U.S. (Marvin-DiPasquale et al. 2009b). MPP rates were likewise an order of magnitude lower than those in the California wetlands but similar to those in the eight U.S. streams. Our finding of higher MeHg concentrations and

MPP rates in floodplain soils compared to stream sediments supported findings from freshwater stream catchments in New York and South Carolina, i.e. that both MeHg concentrations and MPP rates can be much higher in out-of-channel sub-habitats compared to in-channel streambed sediment (Bradley et al. 2011). That study suggested that the stream channel acts more as a conduit for dissolved and particulate THg and MeHg traveling via surface and sub-surface flow from the out-of-channel habitats.

Anoxic microsites

All bed sediments and soils were well-oxygenated when sampled (Table 1). This condition is typical at LEF despite high soil water content. In a nearby catchment, Hall et al. (2013) demonstrated a poor relation between redox status and soil water content; copious water addition failed to induce anoxia. However, soil O₂ concentration at LEF has been shown to fluctuate over both short (day to day) and seasonal time scales, frequently dropping to less than 4%, i.e. less than 20% of atmospheric O₂ (Hall and Silver 2013; Silver et al. 1999). This variability generates spatial (microsites) and temporal reducing conditions where both SRB and FeRB, of which many species are obligate anaerobes, can thrive (Dubinsky et al. 2010; Hall et al. 2016). The occurrence of reducing microsites is supported by previous measurements of pervasive iron and sulfate reduction in these soils irrespective of short-term moisture variability (Hall et al. 2016, 2013), as well as appreciable TRS concentrations, elevated ratios of %Fe(II)/Fe_T, and CH₄ efflux (Table 1). The presence of highly reduced groundwater at a nearby floodplain site (McDowell et al. 1992; McDowell et al. 1996) suggests the possibility of Hg methylation along deeper hydrologic flow paths. However, activity of Fe-reducing microbes (and presumably other anaerobes) appeared to be much greater in microsites at the soil surface, due to increased C limitation with depth (Hall et al. 2016).

Sulfate reducing bacteria

The methylation process has long been thought to be carried out by SRB. However, measured SR rates were low across all study sites (Table 1). If anything, in-situ SR rates in our oxygenated soils were likely lower than those measured in the lab under anoxic conditions, since SRB are strict anaerobes. SR rates at our LEF sites were two orders of magnitude less than similarly assayed freshwater in managed and agricultural wetlands in California (Marvin-DiPasquale et al. 2014) and a diversity of eight stream sites throughout the continental U.S. (Marvin-DiPasquale et al. 2009b). Solid phase TRS was also one to two orders of magnitude less than in both the California wetland and

eight-stream studies. Relative to the other four USGS WEBB watersheds which were concurrently sampled, Río Icacos bed sediment had a median microbial SR rate that was an order of magnitude lower and TRS concentration that was more than 4-fold lower (Table OR2). Further, streambed porewater sulfate concentrations were low (Table 1) and in the range (<30 μmol L⁻¹) that could limit the activity of SRB (Lovley and Klug 1986). Finally, the hill-slope transect lacked the systematic downslope decrease in the porewater SO₄²⁻/Cl⁻ molar ratio one might expect if microbial SR were active and increasing with soil organic and moisture content.

Iron reducing bacteria

Recent advances in molecular biology have demonstrated Hg(II)-methylation capacity in a much more taxonomically diverse array of microbes than previously recognized (Gilmour et al. 2013), including iron-reducing bacteria (FeRB) (Fleming et al. 2006; Kerin et al. 2006). In contrast to SRB, Fe(III)-reducing bacteria are abundant in terrestrial surface soils of the LEF (Dubinsky et al. 2010), and Fe reduction and oxidation occur at significant rates even under non-saturated conditions in this system (Hall et al. 2013). LOI, % Fe(II)_{HCl}, and %Fe(II)/Fe_T each increased downslope from midslope to the floodplain (Table 1), indicating an increase in Fe(III)-reduction with increasing soil organic matter. A corresponding decrease in Fe(III)_{HCl} and Fe(III)_{SRO}, both Fe (III) fractions that are readily available electron acceptors for microbial Fe reduction (Hyacinthe et al. 2006; Lovley and Phillips 1987), imply Fe(III) losses via reductive dissolution in surface horizons over pedogenic timescales.

The evidence that microbial Fe(III)-reduction is a more important terminal carbon flow pathway than microbial sulfate reduction in the sites studied does not necessarily imply that MeHg production was primarily controlled by FeRB. However, an FeRB control is supported by strong correlations of a number of key Hg cycling metrics with Fe cycling metrics. Specifically, MeHg concentration was positively correlated with both %Fe(II)_{HCl} ($r = 0.85$) and % Fe(II)/Fe_T ($r = 0.77$), although %MeHg was not correlated ($p < 0.05$) with these same two (or any) Fe metrics. Thus, while Fe(III)-reduction may be an important microbial process in these Puerto Rican soils/sediments, and metrics for Fe(III)-reduction are correlated with those for MeHg production, the low MeHg concentrations and k_{meth} (and MPP) rates suggest that conditions are sub-optimal for MeHg production.

Demethylation

Considering only MeHg concentrations and k_{meth} (and MPP) rates in assessing Hg cycling within a suite of sites can be

misleading if MeHg degradation plays a significant role in net MeHg production (Marvin-DiPasquale and Agee 2003; Marvin-DiPasquale et al. 2003). In the current study, values of k_{deg} and MDP exceeded corresponding values of k_{meth} and MPP by an order of magnitude (Table 1). Our values of k_{deg} were similar to those measured in Everglades (wetland) flocculent surface sediment (Marvin-DiPasquale and Orem-land 1998) and somewhat lower than San Francisco Bay 0–4 cm surface sediment (Marvin-DiPasquale and Agee 2003), and much lower than those from a Hg-contaminated mining site (Marvin-DiPasquale et al. 2000). While the current results suggest that MeHg degradation exceeded MeHg production in these PR sites, it is difficult to directly compare these ‘potential’ rates due to differences in non-tracer level amendment concentrations and incubation times (6 days for MDP vs 1 day for MPP). However, NMP rates, measured over 6 days of anoxic incubation, suggest limited net positive MeHg production overall (Table 1). Note that as with measurements of microbial SR rates, MeHg production was optimized in the laboratory by the anoxic incubation conditions, in comparison to the more oxic field conditions. Thus, measured NMP rates should be considered upper limits for these substrates. More detail on the methylation experiments is available in the supplemental OR material.

Resolving the paradox

Our resolution of the paradox of high Hg deposition but low biotic uptake in this tropical wet forest rests on two main findings: (1) conditions for MeHg production are sub-optimal, i.e. oxic soil and sediment, thus inhibiting to strict anaerobic bacteria; and (2), potential rates for MeHg degradation are comparatively high. These two factors combine to limit net MeHg production, which results in low biotic Hg uptake. To the extent that there is low but measurable MeHg in the substrate, and low but measurable NMP rates observed under anoxic incubation conditions, there is some potential for MeHg production, likely associated with reduced microsites. However, it is unclear if this limited MeHg production is carried out by SRB that exhibit very low rates, or by FeRB that seem to dominate carbon flow in this setting.

In addition to the low net MeHg production in this system, its high rainfall and associated frequent flushing of watershed soils may limit the residence time of MeHg in the soil for biotic uptake to occur. In the adjacent Mameyes watershed, Scholl et al. (2015) determined from deuterium excess analysis that streamflow was a mix of 75% groundwater and 25% recent (<1 week old) rainfall. The latter component represents shallow flow and the short residence time implies that soils are frequently flushed. Despite the low MeHg/THg ratio in the stream, the high THg and water fluxes resulted in MeHg export that is an order of magnitude greater than the global average

(calculated from Shanley and Bishop (2012)). Thus, the rate of hydrologic flushing of MeHg may keep pace with its net production rate in the soil. Moreover, MeHg in streamwater occurs primarily in the particulate phase (Shanley et al. 2008), which has limited biological availability.

A final factor that may contribute to the low biotic uptake is biomass dilution. Because of the increasing biodiversity toward the tropics (Eldridge 1998), MeHg uptake in the Luquillo forest food web may be distributed across a high faunal biomass so that the concentration at a given trophic level (feeding guild) is lowered. Birds at LEF have high areal biomass density, and energy flows through the avifaunal community at a rate that is at least double that in temperate forests (Wunderle and Arendt 2011). This doubled activity implies a relative dilution of available MeHg by one-half, so biomass dilution may also be a factor contributing to low bird blood Hg in this ecosystem.

Comparison to other tropical studies

Considering that the primary source of Hg in precipitation at this Puerto Rican site is GOM from the global pool present at high altitude, high Hg(II) wet deposition could occur in other tropical settings where there is sufficient landmass to generate high rain-forming convective clouds. However, there are few published studies of Hg in atmospheric deposition or streams in the tropics. Studies on methylation dynamics in the tropics have been more numerous, but most field research has been conducted in lakes and adjacent wetlands in the seasonally flooded floodplains of the Amazon and Paraguay River basins (Achá et al. 2011; Guimarães et al. 2000; Lázaro et al. 2016; Mauro et al. 2002; Roulet et al. 2001). A common thread of these studies is the importance of periphyton in open waters, wetlands, and adjacent wet forests as a methylation hotspot. Indeed, Molina et al. (2010) found that invertebrate food chains based on periphyton were the longest, with the greatest Hg bioaccumulation. As in our study, Correia et al. (2012) hinted that microbes other than SRB may be responsible for methylation in tropical systems. We found no analog in the literature to our methylation study in a forested tropical upland.

The existing literature on Hg in the food web in the American tropics is scant, but shows that unlike this site in Puerto Rico, bioaccumulation of Hg at other tropical sites is comparable or even higher than in temperate latitudes. None of these tropical studies recorded Hg deposition nor investigated methylation processes. Therefore, it is challenging to interpret the sources and processes involved in the trophic transfers of Hg. The Townsend et al. (2013) avian study in Hispaniola was the most comprehensive in terms of number of species, individuals, and habitats sampled, and demonstrated a clear biomagnification of Hg when moving from coastal rain forest to mountain cloud forest. It is unknown

whether this shift is due to increased Hg deposition, increased net methylation, changes in the food web structure, or some combination of these factors. In our study, high Hg deposition poses a potential threat to the biota, but processes acting within LEF work to limit Hg bioaccumulation. Large information gaps remain as to whether elevated atmospheric Hg deposition (as observed in LEF) is widespread in Puerto Rico and the greater tropics, how diverse tropical landscapes cycle atmospherically deposited Hg, and ultimately how much Hg enters tropical food webs and the extent to which Hg poses a risk to human populations. Research should be augmented at existing tropical research sites to link currently uncoordinated investigations of atmospheric deposition, fluvial transport, methylation dynamics, and trophic uptake.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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